Professor Chris Rudd Co-stimulation and Receptors 27th October 2015

Antigen receptor signalling, although essential, is generally insufficient on its own for the activation of T-cells. Instead, T-cells require additional 'co-signals' or 'co-stimulation' that either increase or decrease the T-cell response. They also alter the nature of the immune response by differentially activating T-cell subsets, cytokines and affecting cell viability. Classically, as proposed by Bretscher and Cohn, T-cells require two signals for an optimal T-cell response. Peptides presented to antigen-specific T-cells in the context of MHC molecules deliver signal 1, whereas a co-stimulatory signal delivered by a distinct co-receptor triggers signal 2. CD28 is the best characterised of the co-receptors. CD28 and inducible T-cell co-stimulator (ICOS) generate positive signals, while cytotoxic T-lymphocyte antigen-4 (CTLA-4), programmed death-1 (PD-1), B and T lymphocyte attenuator (BTLA) and T-cell immunoglobulin and mucin-domain-containing molecule-3 (Tim-3) deliver negative signals. The balance of stimulatory and inhibitory signals is important in that they act to to maximize protective immune responses while maintaining immunological tolerance and preventing autoimmunity. A major development has occurred in the treatment of cancer where blocking antibodies to CTLA-4 and PD-1 have been proven effective in the treatment of cancer. This approach has been termed 'immune checkpoint blockade" and was identified by Science magazine in 2013 as the 'Breakthrough of the Year'.

CD28 binds to the ligands B7-1 (CD80) and B7-2 (CD86), is expressed on resting and activated T-cells and provides the optimal signals for interleukin-2 production and proliferation. CD28 can also rescue cells from antigen-induced cell death (AICD) by up-regulating the pro-survival factors Bcl-2 and BcL-XL. CD28 has a small cytoplasmic domain that contains an YMNM motif that binds to phosphatidylinositol 3-kinase (PI 3K) and the adaptor protein Grb-2. PI 3K generates phospholipids, regulates the endocytosis of CD28 and provides survival signals. Grb-2 links the co-receptor to the activation of the pro-inflammatory transcription factor NFκb. A distal tyrosine motif binds to $p56^{lck}$ that in turn is linked to protein kinase theta (PKCθ), a key marker of the immune synapse. Chimeric antigen receptor (CAR) therapy by Carl June and co-workers has used the CD28-PI-3K binding site in the treatment of cancer.

ICOS is a member of the CD28 supergene family, is preferentially expressed on activated Th2 T-cells, binds to the ICOS-ligand and remains present on effector and memory T-cells. ICOS possesses an YMFM motif that binds PI3-kinase, but lacks the asparagine (N) that is needed for Grb-2 binding. Signalling via ICOS provides critical T helper function to B-cells, but does not support interleukin-2 production.

CTLA-4 (CD152) is structurally related to CD28, binds the same ligands CD80/86 with higher avidity and is expressed on activated T-cells. The phenotype of CTLA-4 deficient mice, which develop a massive lymphoproliferative disorder, and the animals die at the age of 3-4 weeks. Several mechanisms have been reported for CTLA-4 inhibition of T-cell activation (i.e. ectodomain competition for CD28 binding to CD80/CD86, down-regulation/removal of CD80/CD86, disruption of CD28 localization at the immunological synapse, modulation of TcR signalling by the phosphatases SH2 domain–containing protein tyrosine phosphatase (SHP-2) and the serine-threonine phosphatase PP2A, as well as interference with the expression or composition of lipid rafts on the surface of T cells).

Like CD28, CTLA-4 contains a small cytoplasmic tail with two tyrosine-based motifs (YVKM and YFIP). Several intracellular proteins bind to the YVKM sequence including PI3K, the phosphatases SHP-2 and PP2A and the clathrin adapter proteins AP-1 and AP-2. CTLA-4 is primarily an intracellular antigen whose surface expression is tightly regulated by restricted trafficking to the cell surface and rapid internalization. CTLA-4 is expressed on the cell surface in activated conventional T-cells, is constitutively expressed on regulatory T-cells (Tregs) and is needed for effective suppressor function.

Programmed death 1 (PD-1) (CD279) is a member of the CD28 supergene family, is expressed on activated T and B cells and binds to ligands for PD-L1 (B7-H1) and PD-L2 (B7-DC). PD-L1 is constitutively expressed on lymphoid and non-lymphoid cells, while PD-L2 is induced on dendritic cells, macrophages and mast cells. Early studies produced conflicting functional data, with two groups showing a position function, and two groups showing a negative function. PD-1 is highly expressed on exhausted (T-cell exhaustion) CD8 T-cells responding to chronic viral infections by LCMV and HIV1. The cytoplasmic tail contains an ITIM (immunoreceptor tyrosine-based inhibition motif) and ITSM (immunoreceptor tyrosine-based switch motif motif) to which the phosphatases SHP-1 and SHP-2

can be recruited upon ligand engagement. Anti-PD-1 or PDL1 blockade has been shown to restore the function of "functionally exhausted" T-cells leading to the elimination of virus' and cancer. Combined anti-CTLA-4 and PD-1 blockade has provided remarkable therapeutic results in eliminating tumours.

B and T lymphocyte attenuator (BTLA) exerts inhibitory effects on B and T lymphocytes, is induced on T-cells during activation, and remains expressed on Th1, but not Th2 cells. BTLA deficient mice show increased humoral responses to T-cell dependent antigens and an increased susceptibility to peptide antigen-induced experimental autoimmune encephalomyelitis (EAE). The unique ligand for BTLA is the herpesvirus entry mediator (HVEM), a member of the TNFR superfamily. The cytoplasmic domain of BTLA contains a Grb-2 binding site, an ITIM and ITSM motif (binding to SHP-1 and SHP-2).

Mucin-domain-containing molecule-3 (**TIM-3)** is preferentially expressed on differentiated Th1 cells and plays an important role in suppressing Th1 effector activation. Galectin-9, a member of the galectin family expressed on lymphocytes and other cell types, has been identified as ligand for Tim-3. Blockade of the Tim-3 pathway leads to an increase in Th1 cell proliferation, cytokine responses and a loss of tolerance.

References

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